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(54) Title: CONTROLLED RELEASE OF THERAPEUTICS BY IN-SITU ENTRAPMENT BY MATRIX CROSS-LINKING

(57) Abstract: The present invention is directed to pharmaceutical compositions, and method for preparing pharmaceutical compositions, comprising a cross-linked matrix physically entrapping at least one therapeutic agent. The matrix is prepared by incubating a solution comprising the at least one therapeutic agent and at least one polymer on which at least two thiol groups are present under conditions that cause cross-linking of the thiol groups to form a matrix entrapping the at least one therapeutic agent. The matrix comprising the at least one therapeutic agent has at least one controlled release in-vivo kinetic profile, and may have additional profiles for the same agent. The matrix may also comprise more than one therapeutic agent, and each additional therapeutic agent may have one or more controlled release in-vivo kinetic profile.

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CONTROLLED RELEASE OF THERAPEUTICS BY IN-SITU ENTRAPMENT BY MATRIX CROSS-LINKING

FIELD OF THE INVENTION

The present invention relates to a method for preparing and pharmaceutical compositions comprising a therapeutic agent entrapped in a cross-linked matrix comprising a polymer on which at least two thiol groups are present. Such matrixes exhibit controlled release kinetic profiles making them suitable for controlled release formulations of various therapeutic agents for the prophylaxis or treatment of conditions and diseases.

BACKGROUND OF THE INVENTION

Therapeutic agents with short half lives, such as most proteins, must be administered by injection at closely repeated intervals to maintain therapeutic benefit, since their in vivo half-lives are minutes to hours. A prominent approach for extending the half-life of a protein to a period of hours or days is to covalently append to it one or more chains of poly(ethylene glycol) (PEG). Appended PEG chains may provide the favorable pharmacologic properties of protection of the underlying protein from immune surveillance and proteolytic enzymes, in addition to the lower rate of clearance from the bloodstream (Davis, S., Abuchowski, A., Park, Y. K. and Davis, F. F. (1981) Clin. Exp. Immunol. 46, 649-652.). However, the successful use of this "pegylation" technology is highly and unpredictably dependent on both the particular protein and the conjugation chemistry. It is also not directly suited to all short-lived therapeutic agents.

Another approach to extending the in vivo lifetime of a therapeutic agent is to administer that agent encapsulated in a sustained release depot. Protein encapsulation processes that require the use of organic solvents or heating potentially physically modify, i.e. denature, a protein drug. A process for preparing protein microparticles by heating in the presence of polymers is described by Woiszwillo et al. (U. S. Patent 5,849,884). A process in which the protein drug is contacted with an organic solvent is described by Zale et al. (U.S. Patent 5,716,644).

Encapsulation processes that require chemical bond formation among the encapsulation reagents might have reactions that unintentionally chemically modify the protein. Thus, this latter method is less favored, since for the example of proteins, which are typically composed of amino acids having a variety of side chain functional groups, chemical modification may impair the pharmacological activity. The same impairment may be imparted to other therapeutic agents.

It is toward the development of a controlled release delivery system for small-molecule drugs, proteins and other therapeutic agents, particularly for those with short in-vivo lifetimes, that the present application is directed.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

In its broadest sense, the present invention is directed to compositions and methods for preparing a cross-linked matrix physically entrapping at least one therapeutic agent

comprising the steps of preparing a solution comprising the at least one therapeutic agent and at least one polymer comprising thiol groups; and then incubating the solution under conditions that cause cross-linking of the thiol groups to form a matrix physically entrapping the at least one therapeutic agent. The matrix comprising the at least one therapeutic agent has at least one controlled release in-vivo kinetic profile, and may have additional profiles for the same agent. The matrix may also comprise more than one therapeutic agent, and each additional therapeutic agent may have one or more controlled release in-vivo kinetic profile.

The therapeutic agent entrapped in the matrix of the present invention is a compound capable of being entrapped and then released in a controlled manner from the matrix. The therapeutic agent is a compound capable of being entrapped and then released in a controlled manner from the matrix. The therapeutic agent may be, by way of non-limiting example, a small-molecule drug, protein, peptide, polysaccharide, polynucleotide, or any other compound that may be entrapped in the matrix of the present invention and subjected to controlled delivery in vivo. By way of non-limiting examples of protein therapeutic agents, proteins include insulin, erythropoietin, α-interferon, growth hormone, or an antibody or antibody fragment. The protein may be a recombinant protein. Non-limiting examples of polysaccharides include sulfated polysaccharides, such as heparin or calcium spirulan. By way of further non-limiting example, polynucleotide therapeutic agents may be antisense oligonucleotides. Other examples include antibiotics, hormones, enzymes, receptors, ligands and cytokines. Vaccines are also embraced by the present invention. A non-limiting example of a small molecule drug includes an anticancer drug, a cardiovascular drug, an antibiotic, an antifungal, an antiviral drug, an AIDS drug, an HIV-1 protease inhibitor, a reverse transcriptase inhibitor, an antinociceptive drug, a hormone, a vitamin, an anti-inflammatory drug, an angiogenesis

drug, and an anti-angiogenesis drug.

In another embodiment of the present invention, the therapeutic agent may be derivatized to increase its molecular weight, such that it may be entrapped by and released from the matrix. The derivatization may be, by way of non-limiting example, polymerization or conjugation to poly(ethylene glycol).

The cross-linking of the polymer on which at least two thiol groups are present may include disulfide bonds, thioether bonds, and combinations thereof. The thiol groups in the thiol-containing polymer may also be present in a protected and/or activated form, such as the S-2-thiopyridine derivative.

The polymer may be a homopolymer or a copolymer. By way of non-limiting example, suitable polymers, which may be derivatized or chemically modified to comprise thiol groups, or functional groups to which a thiol group may be attached, include a polyalkylene oxide such as poly(ethylene glycol) [also known as polyethylene glycol or PEG, polyethylene oxide or PEO], carboxymethylcellulose, dextran, polyvinyl alcohol, N-(2-hydroxypropyl)methacrylamide, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, polypropylene oxide, copolymers of ethylene/maleic anhydride copolymer, polylactide/polyglycolide copolymers, polyaminoacids, copolymer of polyethylene glycol and an amino acid, or polypropylene oxide/ethylene oxide copolymers. In one embodiment, a polymer of the present invention is derived from a poly(ethylene glycol) (PEG) derivative, for example, α,ω-dihydroxy-PEG or α,ω-diamino-PEG, but other derivatives are embraced herein. The polymer comprising thiol groups may be, for example,

a polymer of α,ω-diamino-poly(ethylene glycol) and thiomalic acid; a polymer of α,ω-dihydroxy-poly(ethylene glycol) and thiomalic acid; or a polymer of α,ω-dicarboxy-PEG subunits and lysine wherein the free carboxy groups on the lysine residues are derivatized to form thiol groups.

For example, a poly(ethylene glycol) subunit size for the polymer may be from about 200 to about 20,000 Da; preferably, the subunit size is from about 600 to about 5,000 Da. The polymer of the present invention has from 2 to about 20 thiol groups; preferably from about 3 to about 20 thiol groups, and most preferably, from about 3 to about 8 thiol groups. In one embodiment, the thiol groups on the polymer are sterically hindered, such as is provided when thiomalic acid is used.

Conditions that cause cross-linking of the thiol groups involves reaction of the polymer on which at least two thiol groups are present in the presence of an oxidizing agent, such as by way of non-limiting example, in the presence of molecular oxygen, hydrogen peroxide, dimethylsulfoxide, or molecular iodine. In other embodiments, the cross-linking may be carried out by reaction with a bifunctional disulfide-forming cross-linking agent, or reaction with a bifunctional thioether-forming cross-linking agent. The cross-linking agent may have a molecular weight of about 00 to about 5,000 Da, and may be a polymeric cross-linking agent. By way of non-limiting examples of the latter reactions, a suitable cross-linking agent may be a non-polymeric agent such as 1,4-di-[3',2'-pyridyldithio(propionamido)-butane]; or polymeric cross-linking agents such as α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol); α,ω-divinylsulfone-poly(ethylene glycol); or α,ω-diiodoacetamide-poly(ethylene glycol).

The matrix of the present invention may be provided in a form such as, but not limited to, a gel, microparticles, and nanoparticles.

In another aspect of the present invention, a method is provided for the controlled release of a therapeutic agent in an animal comprising administration to the animal a therapeutically effective amount of the therapeutic agent physically entrapped within a matrix prepared as described above. The matrix may have more than one therapeutic agent, and each therapeutic agent may have one or more controlled release in-vivo kinetic profiles. The matrix may be administered by a route such as but not limited to subcutaneous, oral, intravenous, intraperitoneal, intradermal, subdermal, intratumor, intraocular, intravisceral, intraglandular, intravaginal, intrasinus, intraventricular, intrathecal, intramuscular, and intrarectal. The controlled release of the therapeutic agent from the matrix occurs as a consequence of diffusion from and/or biodegradation of the matrix by one or more in-vivo degradation pathways such as reducing agents, reductases, S-transferases, esterases, peptidases, proteases, non-enzymatic hydrolysis, and thioesterases.

In another embodiment of the present invention, the therapeutic agent in the above-described matrix is prepared in accordance with the above methods and the matrix is formed immediately prior to or during administration to the animal.

In yet another aspect, the present invention is directed to a compositions and pharmaceutical composition consisting of a matrix comprising a therapeutic agent exhibiting at least one first controlled release in-vivo kinetic profile, the matrix comprising at least one cross-linked thiol-containing polymer physically entrapping at least one therapeutic agent. In another

embodiment, the therapeutic agent in the aforementioned matrix has at least one second controlled release in-vivo kinetic profile.

In yet a further aspect of the present invention, the polymer or cross-linking agent may additionally comprise a functional group, such as an amino or carboxyl group. The functional group may be derivatized to provide on the polymer or cross-linking agent a moiety such as but not limited to a label, for example, a contrast/imaging agent, radionuclide, chromophore, fluorophore, or nonradioactive isotope, such that the matrix may be readily located within the body, or the label may be used to monitor degradation of the matrix by detecting a metabolically stable moiety in the urine.

In still a further aspect of the invention, the polymer or cross-linking agent may additionally comprise a functional group for linking a therapeutic agent that will be released in a delayed manner requiring considerable matrix degradation, in comparison to a therapeutic agent not having a covalent linkage to the matrix itself.

These and other aspects of the present invention will be better appreciated by reference to the following drawing and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 depicts the cumulative in-vitro release of fluorescein-labeled bovine serum albumin from a hydrogel matrix of the present invention.

Figure 2 shows the release of the chemokine RANTES from a thiol-containing polymer hydrogel in rabbits.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns pharmaceutical compositions and methods for their preparation which are capable of physically entrapping a therapeutic agent and releasing the therapeutic agent with a controlled release kinetic profile in vivo, such as zero order, pseudo zero order, or first order. The pharmaceutical composition comprises a polymer matrix prepared from polymers bearing thiol moieties, such that the thiol moieties are cross-linked by any of a number of processes to physically entrap the therapeutic agent. As will be described in detail below, the polymer on which at least two thiol groups are present is cross-linked in the presence of the therapeutic agent, thus forming a cross-linked polymer with the therapeutic agent entrapped therein. This is accomplished by using sulfur chemistry for cross-linking the polymer, thereby avoiding reaction of virtually all amino acid and carbohydrate side chains of, for example, a protein therapeutic agent undergoing entrapment in the matrix. Although sulfur chemistry is the basis of the cross-linking used in this invention, disulfide bonds already present in a particular protein would be non-reactive under the cross-linking conditions. Also, the sulfur atom in the thioether side chain of methionine residues in the protein drug would be nonreactive. However, proteins containing free thiol groups (cysteine residues that are not in disulfide linkage), might not be suitable for use in

their native form in this invention, and may need to be derivatized or otherwise protected during the entrapment process. Similar considerations are given to other non-protein therapeutic agents which are used in the present invention.

For long term therapy (days, weeks or months) and/or to maintain the highest possible drug concentration at a particular location in the body, the present invention provides a sustained release depot formulation with the following characteristics: (1) the process used to prepare the matrix does not chemically or physically damage the therapeutic agent, in particular proteins, thereby avoiding protein inactivation or rendering the protein immunogenic; (2) the matrix maintains the stability of a therapeutic agent against denaturation or other metabolic conversion by protection within the matrix until release, which is important for very long sustained release; (3) the entrapped therapeutic agent is released from the depot at a substantially uniform rate, following a kinetic profile, and furthermore, a particular therapeutic agent can be prepared with two or more kinetic profiles, for example, to provide a loading dose and then a sustained release dose; (4) the desired release profile can be selected by varying the components and the process by which the matrix is prepared; and (5) the matrix is nontoxic and degradable.

The cross-linked matrix of the present invention entrapping at least one therapeutic agent is prepared by cross-linking a polymer on which at least two thiol groups are present, by any one of various means, in the presence of the therapeutic agent to be entrapped. Various polymer on which at least two thiol groups are present are suitable for the use herein. The polymer on which at least two thiol groups are present may be prepared, for example, by the reaction or derivatization of a particular polymer that does not contain thiol groups, with a

thiol-containing compound, or a compounds to which thiol moieties may be attached. A polymer may be prepared which has reactive terminal ends or functional groups on the ends of the polymer chain which may be subsequently derivatized to attach thiol groups. A copolymer may be prepared with repeating or alternately repeating thiol groups or functional groups which may be subsequently derivatized to have thiol groups. The extent of derivatization to provide thiol groups may be tailored to the requirements of the matrix to be formed. The foregoing examples of the types of suitable polymers is not intended to be limiting, but to be illustrative of the varieties of polymers and polymer derivatives that may be used in the practice of the invention.

To participate in cross-linking, the polymer has at least two thiol groups to participate in the formation of cross-links. For example, the polymer on which at least two thiol groups are present may have from 2 to about 20 thiol moieties. In a preferred embodiment, the polymer has from 3 to about 20 thiol moieties, and in a most preferred embodiment, the thiol containing polymer has from 3 to about 8 thiol moieties.

Examples of suitable subunit polymer backbones for the preparation of the polymer on which at least two thiol groups are present include both homopolymers or copolymers. By way of non-limiting example, suitable polymer backbones, which may be chemically modified to comprise thiol groups, include a polyalkylene oxide such as poly(ethylene glycol) [also known as polyethylene glycol or PEG, polyethylene oxide or PEO], carboxymethylcellulose, dextran, polyvinyl alcohol, N-(2-hydroxypropyl)methacrylamide, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, polypropylene oxide, copolymers of ethylene/maleic anhydride copolymer, polylactide/polyglycolide copolymers, polyaminoacids, copolymer of

polyethylene glycol and an amino acid, or polypropylene oxide/ethylene oxide copolymers. Such polymers are then derivatized or further polymerized to introduce thiol groups; chemical modification of the polymer may be necessary as a step prior to the further derivatization to incorporate thiol groups. For example, a polymer of the present invention may be derived from a poly(ethylene glycol) (PEG) derivative, for example, α,ω-dihydroxy-PEG or α,ω-diamino-PEG, but other derivatives are embraced herein. The polymer comprising thiol groups may be, for example, a polymer of α,ω-diamino-poly(ethylene glycol) and thiomalic acid; a polymer of α,ω-dihydroxy-poly(ethylene glycol) and thiomalic acid; or a polymer of α,ω-dicarboxy-PEG subunits and lysine wherein the free carboxy groups on the lysine residues are derivatized to form thiol groups. These polymers are only examples of possible choices, as the skilled artisan will be aware of numerouse alternatives. As will be noted below, the selection of the polymer, or combinations thereof, will be guided by the desired properties of the final product, particularly the duration of release of the therapeutic agent and the release kinetics. As will also be noted below, a product of the invention may comprise more than one polymer component in order to provide two or more different release characteristics. Of course, more than one therapeutic agent may be included.

In one particular embodiment, a polymer of the present invention is derived from a poly(ethylene glycol) (PEG) derivative, for example, α,ω-dihydroxy-PEG or α,ω-diamino-PEG, but other derivatives are embraced herein. Examples of such polymers with particular molecular weights include α,ω-dihydroxy-$PEG_{3,400}$; α,ω-dihydroxy-$PEG_{1,000}$; α,ω-diamino-$PEG_{3,400}$; and α,ω-diamino-$PEG_{1,000}$. PEG is known to be a particularly nontoxic polymer. These derivatized PEG subunit polymers are used to prepare the polymer on which

at least two thiol groups are present by derivatization with thiomalic acid. As will be shown in an example below, to prepare the polymer on which at least two thiol groups are present from these reactants, the thiol group of thiomalic acid is first protected by reaction with trityl chloride, to produce trityl-thiomalic acid. Subsequently, the polymer on which at least two thiol groups are present is prepared from the trityl-thiomalic acid and, for example, α,ω-dihydroxy-PEG. Under suitable conditions, a carbodiimide is used to condense the α,ω-dihydroxy-PEG with the protected thiomalic acid. After condensation, the trityl group is removed by treatment with trifluoroacetic acid (TFA).

In another embodiment, thiomalic acid may be replaced with dimercaptosuccinic acid, thereby doubling the number of sites available for cross-linking. Doubling the number of sites for cross-linking results in a gel with smaller pores.

In another example, a polymer of α,ω-dicarboxy-PEG and lysine may be prepared, and subsequently the free carboxy groups on the lysine residues are derivatized to form thiol groups.

These examples are provided by way of illustration only and such methods for adding a thiol group to a polymer are known to one skilled in the art.

In a preferred embodiment using PEG as the subunit for preparing the polymer on which at least two thiol groups are present, the poly(ethylene glycol) subunit size for the polymer may be from about 200 to about 20,000 Da; preferably, the subunit size is from about 600 to about 5,000 Da. As mentioned above, the polymer of the present invention has from 2 to about 20 thiol groups; preferably from about 3 to about 20 thiol groups, and most preferably, from about 3 to about 8 thiol groups.

The thiol groups on the polymer on which at least two thiol groups are present may be sterically hindered. It has been found that a polymer on which at least two thiol groups are present with sterically hindered thiol groups tends to be nonreactive with disulfide bonds in the therapeutic agent, particularly a protein, and thus does not interfere with the intramolecular disulfide bonds in the protein. Furthermore, steric hindrance governs the rate at which reductive cleavage of the polymer occurs in vivo. Thus, for the entrapment of proteins or other therapeutic agents with disulfide bonds, a polymer on which at least two thiol groups are present, sterically hindered thiol groups may be preferred. Such sterically hindered thiol groups are also preferred when increased resistance to reductive cleavage is desired, for example in a longer controlled release formulation. Based on the knowledge of the therapeutic agent and the particular controlled release characteristics desired at the site of administration of the matrix, the skilled artisan will be able to design a matrix with the desired characteristics. Examples of such sterically hindered thiol groups include thiomalate, as used in the above example.

The matrix of the present invention is prepared by cross-linking the polymer on which at least two thiol groups are present in the presence of the therapeutic agent. The cross-linking of the polymer on which at least two thiol groups are present may include disulfide bonds, thioether bonds, and combinations thereof. Other means of covalent bond formation of thiol groups in the thiol-containing polymer to effect cross-linking will be known to the skilled artisan and are considered within the scope and spirit of this invention.

In one example, reaction of the polymer on which at least two thiol groups are present in the presence of an oxidizing agent forms disulfide cross-links. This may be achieved by

molecular oxygen, hydrogen peroxide, dimethyl sulfoxide (DMSO), or molecular iodine. In other embodiments, the cross-linking may be carried out by reaction with a bifunctional disulfide-forming cross-linking agent, or reaction with a bifunctional thioether-forming cross-linking agent. Such cross-linking agents may have a molecular weight of about 100 to about 5,000 Da, and may be a polymeric cross-linking agent.

For example, the PEG-thiomalate polymer described above may be cross-linked with the non-polymeric cross-linking agent 1,4-di-[3',2'-pyridyldithio(propionamido)-butane]. Alternatively, a polymeric cross-linking agent such as α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol); α,ω-divinylsulfone-poly(ethylene glycol); or α,ω-diiodoacetamide-poly(ethylene glycol) may be used. Examples of the cross-linking reaction are provided in the examples below; the skilled artisan will be aware of numerous other agents capable for forming the suitable matrix. As noted above, the selection of the cross-linking agent is guided by the desired characteristics of the matrix product, i.e., the controlled release kinetic profile and the duration of release. These factors, as well as the potential reactivity of the cross-linking agent with reactive moieties on the therapeutic agent, must be taken into consideration in selecting the appropriate polymer, and cross-linking agent in the preparation of the product.

The therapeutic agent entrapped in the matrix of the present invention is a compound capable of being entrapped and then released in a controlled manner from the matrix. A wide variety of both high molecular weight and low molecular weight compounds are suitable, and as will be noted below, a compound not suitable because of its small size may be made suitable by appropriate modification by for example, polymerization or conjugation to a polymer. The

therapeutic agent may be a protein, peptide, polysaccharide, polynucleotide, or any other compound that may be entrapped in the matrix of the present invention and subjected to controlled delivery in vivo. It is noted that a further advantage of the present invention is that the matrix protects the therapeutic agent from degradation or other metabolic processing. The agents may be for the prophylaxis or treatment of a condition or disease, or for the purpose of providing controlled delivery of any suitable agent.

For example, when polymers of the following PEG polymers are prepared with thiomalic acid, and then similarly cross-linked, certain properties of the polymer are obtained. The α,ω-dihydroxy-$PEG_{3,400}$ polymer subunit is conjugated via an ester bond to the thiomalic acid, and the resulting product is loosely cross-linked. Likewise, a loosely cross-linked product is formed from thiomalic acid and α,ω-diamino-$PEG_{3,400}$, the thiomalic acid linked through an amide bond to the PEG subunit. In contrast, α,ω-dihydroxy-$PEG_{1,000}$ linked to thiomalic acid through an ester bond is tightly cross-linking, as is α,ω-diamino-$PEG_{1,000}$, through an amide bond.

Among the examples of suitable therapeutic agents are proteins. This includes proteins, peptides, modified proteins and peptides, and conjugates between proteins or peptides and other macromolecules. The protein may be a recombinant protein. For example, candidate agents include erythropoietin, α-interferon, growth hormone and antibodies. Erythropoietin is administered over long periods to promote the formation of red blood cells, such as in conditions including renal failure or cancer therapy-induced anemia. α-Interferon is used to treat certain viral diseases (e.g. hepatitis) and cancers(e.g. hairy cell leukemia). Growth hormone is used for pituitary dwarfism. These compounds are therapeutically effective for

certain indications when administered at low doses over an extended period of time, making them good candidates for controlled delivery from a depot administration as described herein, as they otherwise are administered by injection.

Another group of suitable protein agents are antibodies and antibody fragments, such as those directed against tumor-specific antigens and against inflammatory response proteins such as tumor necrosis factor and interleukin 1, are additional examples of proteins that may be used in the practice of the present invention. As such products usually require frequent parenteral administration, such as by injection, a matrix with an antibody delivered by controlled release provides convenience. The antibody is protected from biodegradative machinery while in the matrix.

Another example of a class of therapeutic agents are polysaccharides. Examples include sulfated polysaccharides, such as heparin or calcium spirulan. Heparin is an anticoagulant for which long-term therapy is indicated in various hypercoagulation disorders and for prophylactic use. Chronic anticoagulation therapy is indicated, for example, postoperatively to prevent stroke and pulmonary embolism, and in deep vein thrombosis. Calcium spirulan is a potent antiviral
agent against both HIV-1 and HSV-1 (herpes simplex virus) (Hayashi et al., 1996, AIDS Research & Human Retroviruses. 12(15):1463-71).

A further example of suitable therapeutic agents is polynucleotides, such as antisense oligonucleotides. These may be delivered to a particular site within the body using the methods described herein, for sustained delivery to target cells or tissues.

Other examples include antibiotics, hormones, enzymes, receptors, ligands and cytokines.

Another example of a therapeutic agent embraced by the invention herein is a vaccine. Administration to an animal of an immunogen in the matrix of the present invention with the proper controlled release kinetics provides the immune system with an antigen for the development of a humoral and/or cellular response. Indeed, fluid flow carrying the released antigen from a subcutaneous depot of the present invention is through lymphatic tissue where the immune response to that antigen may occur.

It will be noted that the judicious placement of the matrix of the present invention will permit targeted delivery to a particular site within the body, and furthermore, allow a higher concentration of the therapeutic agent to contact a particular site than achievable if the same therapeutic agent is administered systemically. In particular, administration of an agent which induces apoptosis in dysproliferative conditions, such as a tumor, may be performed by the placement (herein termed administration) of the matrix in the proximity of the tumor, thus delivering the therapeutic agent proximal to the tumor. The same strategy is used for proximal delivery of therapeutic agents to other particular body sites or compartments.

In another embodiment of the present invention, the therapeutic agent may be derivatized to increase its molecular weight, such that it may be entrapped by and released from the matrix. The derivatization may be, by way of non-limiting example, polymerization or conjugation to poly(ethylene glycol). Such methods are known to the skilled artisan.

The matrix of the present invention may be provided in a form such as, but not limited to, a

gel, microparticles, and nanoparticles.

In another aspect of the present invention, a method is provided for the controlled release of a therapeutic agent in an animal comprising administration to the animal a therapeutically effective amount of the therapeutic agent in a matrix prepared as described above. The matrix may contain more than one therapeutic agent, and an animal may be administered a single therapeutic agent in the form of more than one matrix, each with a particular controlled release kinetic profile.

Administration of the matrix of the present invention is performed to locate the matrix at a desired site for controlled delivery of the therapeutic agent. This may be to a particular body compartment to which the therapeutic agent has a desired targeted effect, or the matrix may be administered to a particular location wherein controlled release may provide the therapeutic agent for distribution throughout the body or to another site from which the administered site drains. Where a number of appropriate sites are possible, one may be selected from which the matrix may be easily removed. The particular site will be determined by the desired effect of the therapeutic agent.

Non-limiting examples of possible sites for administration of the matrix includes subcutaneous, oral, intravenous, intraperitoneal, intradermal, subdermal, intratumor, intraocular, intravisceral, intraglandular, intravaginal, intrasinus, intraventricular, intrathecal, intramuscular, and intrarectal. It will be seen that certain of these sites provides a site from which systemic distribution of the therapeutic agent may occur, for example, intraperitoneal, subcutaneous, and oral. Certain sites may be selected to provide a target tissue or organ to

which the therapeutic agent's efficacy is desired, such as intratumor, intravaginal, intraglandular, intrathecal, intraventricular, and intraocular. For example, an antitumor agent may be entrapped in the matrix of the present invention and implanted in or near a tumor, for targeted delivery to the tumor.

A subject in whom administration of the pharmaceutical composition of the present invention is preferably a human, but can be any animal. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods and pharmaceutical compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., avian species, such as chickens, turkeys, songbirds, etc., *i.e.*, for veterinary medical use.

The controlled release of the therapeutic agent from the matrix is believed to occur as a consequence of the diffusion from and/or biodegradation of the matrix by one or more in-vivo degradation pathways. While not wishing to be bound by theory, and by which the inventors herein have no duty to disclose, it is believed that degradation of the matrix is achieved by local factors at the site of administration such as reducing agents, for example, glutathione; reductases, S-transferases, esterases, peptidases, proteases, non-enzymatic hydrolysis, and thioesterases. The varied presence of these various degradation agents in particular compartments in the body provides further guidance on selecting the appropriate site for administration, and also in the preparation of a matrix to provide the desired release

kinetics in the presence of the particular degradative machinery at the site.

In another embodiment of the present invention, the therapeutic agent in the above-described matrix is prepared immediately prior to or during administration to the animal. For example, just prior to administration, a solution containing the therapeutic agent and the polymer on which at least two thiol groups are present can be mixed with a solution containing the cross-linking agent. Upon mixture, the cross-linking of the polymer begins to occur, physically entrapping the therapeutic agent. As cross-linking proceeds, the mixture changes from a solution to a gel. The immediately mixed solutions can be administered as a liquid, for example, by subcutaneous injection, wherein the injected liquid continues to cross-link and change into a matrix at the site of administration. This simplifies the administration of a solid or semi-solid matrix.

In yet another aspect, the present invention is directed to a pharmaceutical composition consisting of a matrix comprising a therapeutic agent exhibiting at least one first controlled release in-vivo kinetic profile, the matrix comprising at least one cross-linked polymer on which at least two thiol groups are present entrapping at least one therapeutic agent. In another embodiment, the therapeutic agent in the aforementioned matrix has at least one second controlled release in-vivo kinetic profile. Controlled release in vivo kinetic profiles refer to the particular release characteristics of the therapeutic agent from the matrix to provide therapeutically effective delivery of the therapeutic agent to the body.

The pharmaceutical composition is prepared by cross-linking a polymer on which at least two thiol groups are present, by any one of various means, in the presence of the therapeutic agent

to be entrapped. Various polymer on which at least two thiol groups are present are suitable for the use herein. The polymer on which at least two thiol groups are present may be prepared, for example, by the polymerization of a particular polymer subunit that does not contain thiol groups, with a thiol-containing compound, thus forming a larger polymer. To participate in cross-linking, the polymer on which at least two thiol groups are present has at least two thiol groups per polymer to participate in the formation of cross-links. For example, the polymer on which at least two thiol groups are present may have from 2 to about 20 thiol moieties. In a preferred embodiment, the polymer has from 3 to about 20 thiol moieties, and in a most preferred embodiment, the thiol containing polymer has from 3 to about 8 thiol moieties.

Examples of suitable subunit polymers for the preparation of the polymer on which at least two thiol groups are present include both homopolymers or copolymers. By way of non-limiting example, suitable polymers, which may be chemically modified to comprise thiol groups, include poly(ethylene glycol) [also known as polyethylene glycol or PEG, polyethylene oxide or PEO], carboxymethylcellulose, dextran, polyvinyl alcohol, N-(2-hydroxypropyl)methacrylamide, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, polypropylene oxide, copolymers of ethylene/maleic anhydride copolymer, polylactide/polyglycolide copolymers, polyaminoacids, copolymer of polyethylene glycol and an amino acid, or polypropylene oxide/ethylene oxide copolymers. Such polymers are then derivatized or further polymerized to introduce thiol groups; chemical modification of the polymer may be necessary as a step prior to the further derivatization to incorporate thiol groups. The polymer comprising thiol groups may be, for example, a polymer of α,ω-diamino-poly(ethylene glycol) and thiomalic acid; a polymer of

α,ω-dihydroxy-poly(ethylene glycol) and thiomalic acid; or a polymer of α,ω-dicarboxy-PEG subunits and lysine wherein the free carboxy groups on the lysine residues are derivatized to form thiol groups. These polymers are only examples of possible choices, as the skilled artisan will be aware of numerous alternatives. As will be noted below, the selection of the polymer, or combinations thereof, will be guided by the desired properties of the final product, particularly the duration of release of the therapeutic agent and the release kinetics. As will also be noted below, a product of the invention may comprise more than one polymer component in order to provide two or more different release characteristics. Of course, more than one therapeutic agent may be included.

In one particular embodiment, a polymer of the present invention is derived from a poly(ethylene glycol) (PEG) derivative, for example, α,ω-dihydroxy-PEG or α,ω-diamino-PEG, but other derivatives are embraced herein. PEG is known to be a particularly nontoxic polymer. These derivatized PEG subunit polymers are used to prepare the polymer on which at least two thiol groups are present by derivatization with thiomalic acid. As will be shown in an example below, to prepare the polymer on which at least two thiol groups are present from these reactants, the thiol group of thiomalic acid is first protected by reaction with trityl chloride, to produce trityl-thiomalic acid. Subsequently, the polymer on which at least two thiol groups are present is prepared from the trityl-thiomalic acid and, for example, α,ω-dihydroxy-PEG. Under suitable conditions, a carbodiimide compound is used to condense the α,ω-dihydroxy-PEG with the protected thiomalic acid. After condensation, the trityl group is removed by treatment with trifluoroacetic acid (TFA).

In another example, a polymer of α,ω-dicarboxy-PEG and lysine may be prepared, and

subsequently the free carboxy groups on the lysine residues are derivatized to form thiol groups (Huang, S.-Y., Pooyan, S., Wang, J., Choudhury, I., Leibowitz, M. J. and Stein, S. A Polyethylene Glycol Copolymer for Carrying and Releasing Multiple Copies of Cysteine-Containing Peptides. Bioconjugate Chemistry 9, 612-617, 1998). These examples are provide by way of illustration only and such methods for adding a thiol group to a polymer are known to one skilled in the art.

In a preferred embodiment using PEG as the subunit for preparing the polymer on which at least two thiol groups are present, the poly(ethylene glycol) subunit size for the polymer may be from about 200 to about 20,000 Da; preferably, the subunit size is from about 600 to about 5,000 Da. As mentioned above, the polymer of the present invention has from 2 to about 20 thiol groups; preferably from about 3 to about 20 thiol groups, and most preferably, from about 3 to about 8 thiol groups.

The thiol groups on the polymer on which at least two thiol groups are present may be sterically hindered. It has been found that a polymer on which at least two thiol groups are present with sterically hindered thiol groups tends to be nonreactive with disulfide bonds in the therapeutic agent, particularly a protein, and thus does not interfere with the intramolecular disulfide bonds in the protein. Furthermore, steric hindrance governs the rate at which reductive cleavage of the polymer occurs in vivo. Thus, for the entrapment of proteins or other therapeutic agents with disulfide bonds, a polymer on which at least two thiol groups are present with a sterically hindered thiol groups may be preferred. Such sterically hindered thiol groups are also preferred when increased resistance to reductive cleavage is desired, for example in a longer controlled release formulation. Based on the

knowledge of the therapeutic agent and the particular controlled release characteristics desired at the site of administration of the matrix, the skilled artisan will be able to design a matrix with the desired characteristics. Examples of such sterically hindered thiol groups include thiomalate, as used in the above example.

The matrix of the present invention is prepared by cross-linking the polymer on which at least two thiol groups are present in the presence of the therapeutic agent. The cross-linking of the polymer on which at least two thiol groups are present may include disulfide bonds, thioether bonds, and combinations thereof. In one example, reaction of the polymer on which at least two thiol groups are present in the presence of an oxidizing agent forms disulfide cross-links. This may be achieved by molecular oxygen, hydrogen peroxide, dimethylsulfoxide, or molecular iodine. In other embodiments, the cross-linking may be carried out by reaction with a bifunctional disulfide-forming cross-linking agent, or reaction with a bifunctional thioether-forming cross-linking agent. Such cross-linking agents may have a molecular weight of about 100 to about 5,000 Da, and may be a polymeric cross-linking agent.

For example, the PEG-thiomalate polymer described above may be cross-linked with the cross-linking agent 1,4-di-[3',2'-pyridyldithio(propionamido)-butane]. Alternatively, a polymeric cross-linking agent such as α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol); α,ω-divinylsulfone-poly(ethylene glycol); or α,ω-diiodoacetamide-poly(ethylene glycol) may be used. Examples of the cross-linking reaction are provided in the examples below; the skilled artisan will be aware of numerous other agents capable of forming the suitable matrix. As noted above, the selection of the cross-linking agent is guided by the desired characteristics of the matrix product, i.e., the controlled release kinetic profile and the

duration of release. These factors, as well as the potential reactivity of the cross-linking agent with reactive moieties on the therapeutic agent, must be taken into consideration in selecting the appropriate polymer, and cross-linking agent in the preparation of the product.

The therapeutic agent entrapped in the matrix of the present invention is a compound capable of being entrapped and then released in a controlled manner from the matrix. A wide variety of both high molecular weight and low molecular weight compounds are suitable, and as will be noted below, a compound not suitable because of its size may be made suitable by appropriate modification by for example, polymerization or conjugation to a polymer. The therapeutic agent may be a protein, peptide, polysaccharide, polynucleotide, or any other compound that may be entrapped in the matrix of the present invention and subjected to controlled delivery in vivo. It is noted that a further advantage of the present invention is that the matrix protects the therapeutic agent from degradation and other metabolic processing.

Among the examples of suitable therapeutic agents is proteins. This includes proteins, peptides, modified proteins and peptides, and conjugates between proteins or peptides and other macromolecules. The protein may be a recombinant protein, or one prepared synthetically, such as by solid phase synthesis. By way of non-limiting examples, candidate agents include erythropoietin, α-interferon, and growth hormone. Each of these examples is described above. These compounds are therapeutically effective for certain indications when administered at low doses over an extended period of time, making them good candidates for controlled delivery from a depot administration as described herein, as they otherwise are administered by injection. Another group of suitable agents are antibodies and antibody fragments, such as polyclonal, monoclonal, chimeric, single chain, and Fab fragments. Non-

limiting examples are as described above.

Another example of a class of therapeutic agents are polysaccharides. Examples include sulfated polysaccharides, such as heparin or calcium spirulan. The therapeutic and prophylactic utilities of these non-limiting examples of suitable compounds are described above.

A further example of suitable therapeutic agents is polynucleotides, such as transgenic vectors, gene therapy agents and antisense oligonucleotides. These may be delivered to a particular site within the body using the methods described herein, for sustained delivery to target cells or tissues. Such gene therapy agents include but are not limited to a gene encoding a particular protein or polypeptide domain fragment either as a naked plasmid or introduced in a viral vector. Such vectors include, for example, an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like, including retroviral vectors. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. Defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, a particular tissue can be specifically targeted.

Other examples of agents, including vaccines, are those as described above.

In another embodiment of the present invention, the therapeutic agent may be derivatized to increase its molecular weight, such that it may be entrapped by and released from the matrix.

The derivatization may be, by way of non-limiting example, polymerization or conjugation to poly(ethylene glycol). A suitable drug carrier may be constructed, for example, from linear poly(ethylene glycol) (PEG) subunits linked by amino acid subunits. The side chains of the amino acid subunits may be used for attachment of multiple copies of the drug (and other groups as well, such as a label) to the polymer. These PEG-based polymers have been found to be biocompatible. Such methods are known to one skilled in the art.

In yet a further aspect of the methods and pharmaceutical compositions of the present invention, the polymer or cross-linking agent may additionally comprise a functional group, such as an amino or carboxyl group. The functional group may be derivatized to provide on the polymer or cross-linking agent a moiety such as a label, for example, a contrast/imaging agent, a radionuclide, a chromophore, a fluorophore, or a nonradioactive isotope, such that the matrix may be readily located within the body, or the label may be used to monitor degradation of the matrix by detecting a metabolically stable moiety in the urine. The label may be chemically attached to the functional group by, for example, carbodiimide activation or use of a homobifunctional or heterobifunctional cross-linking agent. Examples of contrast/imaging agents include F-19 for MRI, I-126 for X-ray and Tc-99m for radioscintigraphy.

In an typical example of the preparation of a matrix of the invention, the first step is the synthesis of a polymer on which at least two thiol groups are present. In the case of the amide-linked polymer of α,ω-diamino-PEG with thiomalic acid, the thiomalic acid is first protected as (S-trityl)-thiomalic acid, as follows. Equimolar quantities of α,ω-diamino-PEG (MW 3,400, Shearwater Polymers) and (S-trityl)-thiomalic acid were dissolved in methylene

chloride, and 3.5 equivalents of 1,3-diisopropylcarbodiimide (DIPC, Aldrich) was added to carry out a direct polycondensation at room temperature with 0.5 equivalent of 4-(dimethylamino)-pyridine (DMAP, Aldrich) and p-toluenesulfonic acid monohydrate (PTSA, Aldrich) as catalysts. The reaction mixture was precipitated with cold ethyl ether to obtain a white polymer product, which was treated with 100% trifluoroacetic acid for 2 hours to remove the protecting trityl groups from the polymer. The deprotected polymer was precipitated in cold ethyl ether, washed 5 times with ether and dried under vacuum. The molecular weight of the resulting PEG-thiomalic acid polymer was measured by size exclusion chromatography, using PEGs of defined molecular weights (Shearwater Polymers) for calibration of the column.

These polymers are then to be used for the preparation of the matrix entrapping the therapeutic agent. As mentioned above, use of polymers made from the smaller PEG subunits would result in a matrix having more closely spaced cross-links, resulting in a slower rate of diffusion of encapulated conjugate out of the matrix. Amide bonds, resulting from use of the diamino-PEGs, are expected to be considerably more stable *in vivo* than are ester bonds, which corresponds to a lower rate of degradation of the matrix *in vivo*.

For matrix formation, a preferred cross-linking reagent is α,ω-divinylsulfone-PEG (Shearwater Polymers). The vinylsulfone functional group reacts readily and specifically with thiol groups on the matrix-forming polymer, but will not react with disulfide bonds, such as present in a protein with disulfide bonds. As mentioned above, the possibility of cleavage of any disulfide bond in the therapeutic agent can be minimized or essentially prevented by providing steric hindrance to the disulfide linkage in the thiol-containing polymer.

Another factor influencing the release rate of the therapeutic agent is the size and the shape of the matrix depot. The greater the ratio of surface area to volume, the shorter the duration of release. For example, a sheet-like depot would be expected to release the encapsulated agent much faster than would a spherical depot of the same mass. One large sphere would release the agent more slowly than would many small spheres of the same total mass. The selection of the size and shape of the matrix will be readily determinable by a skilled artisan based on desired characteristics of release of the particular therapeutic agent.

As mentioned above, the matrix may be administered just after mixing the polymer on which at least two thiol groups are present with the cross-linking agent, in the presence of the therapeutic agent, such that the mixture may be injected in liquid form but the matrix solidifies into the cross-linked form soon thereafter. Fox example, to practice this aspect of the invention, a dual-syringe pump may be used for making and administering the mixture. For example, one syringe will be filled with 0.5 ml of matrix-forming polymer and the therapeutic agent, while the other syringe will be filled with 0.5 ml of the cross-linker solution (or the therapeutic agent may be mixed in this syringe), both at the equivalent normality. The concentrations selected for these two solutions will be that appropriate to create the matrix with the appropriate controlled release kinetic profile. The pump will be set at a constant flow rate (e.g. 0.1 ml/min). The two solutions will be mixed in a tee-fitting and the mixture will be injected. The mixture becomes viscous as it flows through teflon tubing for a specified time. The mixed solution may be injected to the site of administration, whereupon the solution polymerizes into a hydrogel matrix.

More simply, all components can be mixed in one syringe just prior to administration. The

rate at which the gel forms by the crosslinking reaction and is no longer suitable for injection is preferably in a time frame of a few minutes. This rate may be controlled by the type of functional group on the cross-linking reagent and by the pH of the reaction, being slower at pH 6 compared with pH 7.

With regard to the administration of the matrix as described above, in one embodiment, may comprise 100 injections of 1 microliter each, perhaps repeated at multiple sites around the body, whereby the number and volume of the injections corresponds to a particular pharmacokinetic profile. As noted above, the fluid would be a partially cross-linked viscous matrix as it enters the skin, thereby already entrapping the drug. The microparticles, perhaps uniformly sized at 1 cubic millimeter (about 1 microliter), would harden within minutes as the cross-linking reaction goes to completion. Alternatively, a single needle injection may be used to produce a subcutaneous depot that may be easier to remove surgically in case of an adverse reaction to the depot or the drug.

Factors such as the size and shape of the matrix, the concentration and amount of the therapeutic agent entrapped therewithin, the extent of cross-linking of the polymer on which at least two thiol groups are present and the susceptibility of the polymer and cross-links to biodegradative machinery contribute to the pharmacokinetic profile of the therapeutic agent, the longevity of the matrix, among other factors. Each therapeutic agent will require a particular set of factors to provide the matrix with the correct profile for therapeutic use. In particular, the molecular weight and physical interaction between the agent and the polymers comprising the matrix will participate in the profile. For the practice of the invention, a particular set of preparation and operating conditions will be established for each therapeutic

agent and, in more particular, the desired controlled release profile for that agent. It is well within the realm of the skilled artisan, based on the teaching herein, to determine the matrix components and other factors in the preparation of a suitable range of conditions for preparing a matrix for a particular therapeutic agent which exhibits the desired profile.

Further to the typical procedure described above for the preparation of the matrix of the present invention, variables for the protein solution include but are not limited to protein concentration, pH, salt content and presence of other excipients and stabilizers. The protein may be modified, such as by pegylation, to increase its size and, thereby, decrease its release rate.

In a further aspect of the present invention, a particular release rate may be achieved using a mixture of two or more starting polymer subunits to prepare the thiol-containing polymer or using a mixture of two or more polymers during the cross-linking/entrapment process. A delayed release product may be prepared by first entrapping the protein using an ester-type polymer, followed by coating or encapsulating these resulting particles using an amide-type polymer. The desired release kinetics for the final product may be achieved by administering to the patient a blend of two or more differently and separately cross-linked, entrapped protein preparations. Other means for making a product with a desired release profile will be apparent to the skilled artisan based on the teachings herein and should be considered to be within the scope and spirit of the present invention. As mentioned above, for any particular matrix, the release rate must be determined empirically in vivo, since it is dependent on many factors, including the size of the protein, diffusion from the matrix and the rate of degradation of the cross-linked polymer matrix due to the action of esterases, peptidases and reducing

agents at the site of the depot. What is essential to and definitive of the present invention is that gel formation proceeds in situ without any chemical reactions occurring with the therapeutic agent.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

Example 1

Preparation of an amide-linked polymer on which at least two thiol groups are present

I. Preparation of thiol group-protected thiomalic acid (trityl-thiomalic acid). One equivalent of thiomalic acid and 3 equivalents of trityl chloride were dissolved in dimethylformamide (DMF). The reaction was carried out at room temperature with stirring overnight. The reaction mixture was loaded onto a silica gel column and the eluted fractions containing trityl-thiomalic acid were collected and evaporated to dryness. The product was characterized by NMR.

II. Synthesis of amide-linked polymers from α,ω-diamino-PEG and trityl-thiomalic acid. Equimolar quantities of α,ω-diamino-PEG (MW 3,400; Shearwater Polymers, Inc. Huntsville, AL) and thiol group-protected thiomalic acid as prepared above were dissolved in methylene chloride, and 3.5 equivalents of 1,3-diisopropylcarbodiimide (DIPC, Aldrich,

Milwaukee, WI) were added to carry out a direct polycondensation at room temperature with 0.5 equivalent of 4-(dimethylamino)-pyridine (DMAP, Aldrich, Milwaukee, WI) and p-toluenesulfonic acid monohydrate (PTSA, Aldrich, Milwaukee, WI) as catalyst. The reaction mixture was precipitated with cold ethyl ether to obtain a white polymer product. The polymer was treated with 100% trifluoroacetic acid (TFA) for 2 hours to remove the protecting trityl groups from the polymer pendant chain. The deprotected polymer was precipitated in cold ethyl ether, washed 5 times with ether and dried under vacuum. The molecular weight of the resulting PEG-thiomalic acid polymer was measured by size exclusion chromatography (SEC).

Example 2

Preparation of an ester-linked polymer on which at least two thiol groups are present

Polymerization of thiomalic acid with α,ω-dihydroxy-PEG. Equimolar quantities of α,ω-dihydroxy-PEG and thiomalic acid are dissolved in methylene chloride, and 3.5 equivalent of 1,3-diisopropylcarbodiimide (DIPC, Aldrich, Milwaukee, WI) are added to carry out a direct polycondensation at room temperature with 0.5 equivalent of 4-(dimethylamino)-pyridine (DMAP, Aldrich, Milwaukee, WI) and p-toluenesulfonic acid monohydrate (PTSA, Aldrich, Milwaukee, WI) as catalyst. The reaction mixture is precipitated with cold ethyl ether to obtain
the polymer product.

Example 3

Preparation of an ester-linked-thiol-protected thiol-containing polymer

Polymerization of thiol-protected thiomalic acid with α,ω-dihydroxy-PEG. Equimolar quantities of α,ω-dihydroxy-PEG and thiol group-protected thiomalic acid are dissolved in methylene chloride, and 3.5 equivalents of 1,3-diisopropylcarbodiimide (DIPC, Aldrich, Milwaukee, WI) are added to carry out a direct polycondensation at room temperature with 0.5 equivalent of 4-(dimethylamino)-pyridine (DMAP, Aldrich, Milwaukee, WI) and p-toluenesulfonic acid monohydrate (PTSA, Aldrich, Milwaukee, WI) as catalyst. The reaction mixture is precipitated with cold ethyl ether to obtain the protected polymer product. The polymer is treated with 100% TFA for 2 hours to remove the protecting trityl groups from the polymer pendant chain.

Example 4

Preparation of fluorescein-labeled bovine serum albumin

Five mg bovine serum albumin (BSA, Sigma, St. Louis, MO) is dissolved in 5 mL 0.2 M borate buffer pH 8.5. Five mg fluorescein isothiocyanate (FITC, Sigma, St. Louis, MO) is dissolved in 200 uL acetone. The two solutions are mixed and reacted at room temperature (25°C) for 2 hours with stirring. Use Microcon 50 ultrafiltration (MWCO 50,000 Da, Amicon, Inc.,
Beverly, MA) to remove excess FITC; the retentate is washed by ultrafiltration with PBS, pH 7.4 until no free FITC appears in the filtrate. The FITC-labeled BSA is collected and stored at 4°C.

Example 5

Entrapment of fluorescein-BSA by cross-linking of thiol-containing polymers

In an example of the cross-linking by oxygen of the amide-linked PEG described in Example 1, two mg polymer as prepared in Example 1, was dissolved in 100 microliters of carbonate buffer pH 8.5. Ten μL of FITC-BSA (about 50 μg) is added, and the mixture bubbled with filtered compressed air until the hydrogel forms. The FITC-BSA is entrapped in the resultant hydrogel.

In an example of the cross-linking by DMSO of amide-linked polymers, 2.5 mg polymer prepared in Example 1 is dissolved in 70 μL PBS, pH 7.4. Ten μL of FITC-BSA (about 50 μg) is added. The solution is mixed, and 20 μL DMSO added. The mixture is allowed to stand at room temperature (25°C) until a hydrogel forms, entrapping the FITC-BSA.

In a further example of the entrapment of fluorescein-BSA by cross-linking of amide-linked polymers using a cross-linking agent, 3 mg polymer prepared in Example 1 and 1 mg BSA are dissolved in 90 μL PBS, pH 7.4. Six μL of FITC-BSA (about 100 μg) is added, with mixing. Five μL (0.3 mg) of 1,4-di-[3',2'-pyridyldithio(propionamido)butane] (DPDPB, Pierce Chemical Co.,Rockford, IL) in DMSO is added. The mixture is allowed to stand at room temperature (25°C) until a hydrogel forms, entrapping the FITC-BSA.

In another example, entrapment of fluorescein-BSA by cross-linking of amide-linked polymers using a polymeric cross-linking agent. Three mg polymer prepared in Example 1 and 1 mg BSA are dissolved in 90 μL PBS, pH 7.4. Six μL of FITC-BSA (about 100 ug) is added, with mixing. Three mg α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol) [PEG-$(OPD)_2$] (MW 3,400 Da; Shearwater Polymers, Inc., Huntsville, AL) is added to the

solution. The mixture is allowed to stand at room temperature (25°C) until hydrogel forms, entrapping the FITC-BSA.

Example 6

Entrapment of fluorescein-bovine serum albumin in cross-linked hydrogel.

Two mg polymer on which at least two thiol groups are present as prepared in Example 1 is dissolved in 50 mL of PBS, pH 7.4. 0.6 mg PEG-$(VS)_2$ (MW 2000 Da, Shearwater Polymers, Inc., Huntsville, AL) is dissolved in 40 mL of PBS, pH 7.4 and 10 mL of fluorescein-BSA (about 200 mg). The two solutions are mixed thoroughly. The final concentration of the polymer on which at least two thiol groups are present solution before the hydrogel formation was 2% w/v (2 mg in 100 microliters). The mixture was allowed to stand at room temperature (25°C) until the hydrogel forms. The amount of the protein entrapped in the hydrogel was about 93%, based on fluorescence measurement.

Example 7

Entrapment of fluorescein-bovine serum albumin in a densely cross-linked hydrogel.

Four mg of the polymer on which at least two thiol groups are present prepared in Example 1 is dissolved in 50 mL of PBS, pH 7.4. 0.6 mg of α,ω-divinylsulfone-PEG [PEG-$(VS)_2$] (MW 2000 Da, Shearwater Polymers, Inc., Huntsville, AL) is dissolved in 40 mL of PBS, pH 7.4 and 10 mL of fluorescein-BSA (about 200 mg). The two solutions are mixed thoroughly. The final concentration of the polymer on which at least two thiol groups are present solution before the hydrogel formation was 4% w/v (4 mg in 100 microliters). The mixture is allowed

to stand at room temperature (25°C) until hydrogel forms. The amount of the protein entrapped in the hydrogel was about 97%, based on fluorescence measurement of the excess liquid after hydrogel formation.

Example 8

Release of fluorescein-bovine serum albumin from a hydrogel polymer on which at least two thiol groups are present

The hydrogel formed in Example 6 (2% hydrogel) or Example 7 (4% hydrogel) was first washed with 3x200 mL of PBS, pH 7.4 to remove any non-entrapped proteins. To conduct the release study, 200 mL of PBS, pH 7.4 was added and allowed to incubate with the hydrogel for pre-selected time period. The supernatant was removed from the hydrogel for fluorescence measurement, and fresh PBS added for next incubation.

Based on fluorescence measurements of each collected release sample, the release profile of fluorescein-BSA from the polymer on which at least two thiol groups are present hydrogel in PBS, pH 7.4 at 25 C is shown in Figure 1. A controlled release of the entrapped BSA is shown over a period of about three weeks.

Example 9

Entrapment and *in vivo* release study of chemokine from thiol containing polymer hydrogel in rabbits.

Forty mg of matrix-forming polymer on which at least two thiol groups are present as

prepared in Example 1 is dissolved in 250 microliters of chemokine solution containing about 0.5 mg of chemokine, to which another 250 microliters of saline was added to a final volume of 500 microliters. Twelve mg of cross-linker, PEG-$(VS)_2$ (MW 2000 Da, Shearwater Polymers, Inc., Huntsville, AL) was dissolved in 500 microliters of saline. The two solutions were mixed thoroughly in a 3 cc syringe. The final concentration of the polymer solution before the hydrogel formation was 4% w/v (40 mg in 1 mL). The solution is injected subcutaneously (SC) in the back ear area of a New Zealand White rabbit (about 2.5 kg in weight). A round-shaped polymer depot with chemokine entrapped is formed *in situ* at the injection site. The rabbit is bled at 0, 1, 2, 4, 6 hours and then day 2, 3, 8, 17, and 21. Plasma is collected using EDTA as an anticoagulant by centrifuging serum at 0 °C within 10 minutes of the collection.

An Enzyme Linked Immunosorbent Assay (ELISA) (Human RANTES ELISA kit, R&D Systems, Inc., Minneapolis, MN) is used for the quantitative determination of chemokine concentration in collected plasma by following the provided assay procedure. The release profile of chemokine from thiol containing polymer hydrogel is shown in Figure 2.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the

appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method of preparing a thioether cross-linked hydrogel drug depot, said method comprising: preparing a solution comprising a therapeutic agent and a polymer system capable of forming a thioether cross-linked hydrogel matrix, said polymer system comprising a first polymer having a plurality of thiol groups, and a second polymer having two or more vinyl sulfone groups; and forming thioether linkages between said thiol groups and said vinyl sulfone groups so as to form a thioether cross-linked hydrogel matrix having said therapeutic agent entrapped therein.

2. The method of claim 1, wherein said first polymer comprises a polyalkylene oxide.

3. The method of claim 1, wherein said second polymer comprises a polyalkylene oxide.

4. The method of any one of claims 2 and 3, wherein said polyalkylene oxide comprises a homopolymer, a copolymer or a combination thereof.

5. The method of any one of claims 2 and 3, wherein said polyalkylene oxide comprises polyethylene glycol or a derivative thereof.

6. The method of claim 1, wherein one or more of said first and second polymers comprises one or more biodegradable linkages.

7. The method of claim 1, wherein said plurality of thiol groups is between 2 and 20.

8. The method of claim 1, wherein said second polymer is a bifunctional vinyl sulfone functionalized cross-linking agent.

9. The method of claim 1, wherein said forming is in vivo.

10. The method of claim 9, further comprising releasing a therapeutically effective amount of said therapeutic agent from said thioether cross-linked hydrogel matrix over a time course of three or more days.

11. The method of claim 10, wherein said time course is five or more days.

12. The method of claim 10, wherein said time course is ten or more days.

13. The method of claim 10, wherein said time course is fifteen more days.

14. The method of claim 10, wherein said time course is twenty or more days.

15. The method of claim 10, wherein said releasing comprises a controlled release profile comprising an initial bolus release profile followed by a release profile selected from the group consisting of zero order, pseudo zero order, and first order.

16. The method of claim 1, wherein said therapeutic agent is selected from the group consisting of small organic molecule, nucleic acid, peptide, polypeptide, protein, carbohydrate, vaccine, adjuvant, and lipid.

17. A method of administering a therapeutic agent to a mammal, said method comprising: preparing a solution comprising a hydrogel forming polymer having two or more thiol groups, a cross-linker comprising two or more vinyl sulfone groups, and a therapeutic amount of drug; and injecting said mammal with said solution whereby a hydrogel drug depot is formed at the site of injection having said drug temporarily entrapped therein.

18. A method of administering a therapeutic agent to a mammal, said method comprising: preparing a solution comprising a hydrogel forming polymer having two or more vinyl sulfone groups, a cross-linker comprising two or more thiol groups, and a therapeutic amount of drug; and injecting said mammal with said solution whereby a hydrogel drug depot is formed at the site of injection having said drug temporarily entrapped therein.

19. The method of any one of claims 17 and 18, wherein said cross-linker comprises a hydrogel forming polymer.

20. The method of anyone of claims 17 and 18, further comprising releasing a therapeutically effective amount of said therapeutic agent from said hydrogel drug depot over a time course of three or more days.

21. The method of anyone of claims 17 and 19, wherein said injecting is subcutaneous.

22. A hydrogel drug depot comprising a therapeutic agent entrapped within a polymer

matrix comprising a thioether cross-linked hydrogel matrix.

23. The hydrogel drug depot of claim 22, wherein said hydrogel matrix comprises a polyalkylene oxide.

24. The hydrogel drug depot of claim 24, wherein said polyalkylene oxide is a homopolymer, copolymer or combination thereof of polyethylene glycol or derivative thereof.

25. The hydrogel drug depot of claim 23, wherein polymer matrix comprises a controlled release kinetic profile characterized by release of a therapeutically effective amount of said therapeutic agent from said thioether cross-linked hydrogel matrix over a time course of three or more days.

26. The hydrogel drug depot of claim 25, wherein said time course is five or more days.

27. The hydrogel drug depot of claim 25, wherein said time course is ten or more days.

28. The hydrogel drug depot of claim 25, wherein said time course is fifteen or more days.

29. The hydrogel drug depot of claim 25, wherein said time course is twenty or more days.

30. The hydrogel drug depot of claim 25, wherein said controlled release kinetic profile comprises an initial bolus release profile followed by a release profile selected from the group consisting of zero order, pseudo zero order, and first order.

31. The hydrogel drug depot of claim 22, wherein said therapeutic agent is selected from the group consisting of small organic molecule, nucleic acid, peptide, polypeptide, protein, carbohydrate, vaccine, adjuvant, and lipid.

32. The hydrogel drug depot of claim 22, wherein said thioether cross-linked hydrogel matrix is formed by cross-linking a first polymer containing two or more thiol groups with a second polymer containing two or more vinyl sulfone groups.

33. The hydrogel drug depot of claim 32, wherein said first polymer comprises a molecular weight of 200 to 20,000 Daltons.

34. The hydrogel drug depot of claim 32, wherein said second polymer comprises a molecular weight of 100 to 5,000 Daltons.

35. The hydrogel drug depot of claim 32, wherein said first polymer comprises between 2 and 20 thiol groups.

36. The hydrogel drug depot of claim 32, wherein said first and second polymers are in a defined molar ratio for controlling the controlled release kinetic profile of said hydrogel drug depot.

37. The hydrogel drug depot of claim 22, wherein said thioether cross-linked hydrogel matrix comprises one or more biodegradable linkages.

38. A hydrogel drug depot system comprising a compound of interest, a first polyalkylene oxide polymer containing two or more thiol groups, and a second polyalkylene oxide polymer containing two or more vinyl sulfone groups that are capable of covalently bonding to one another to form a thioether cross-linked hydrogel matrix, said hydrogel drug depot system comprising a controlled release kinetic profile characterized by sustained release of said compound of interest from said thioether cross-linked hydrogel matrix over a time course of three or more days.

39. The hydrogel drug depot of claim 38, wherein said polyalkylene oxide is polyethylene glycol or a derivative thereof.

40. The hydrogel drug depot of claim 38, wherein said hydrogel matrix comprises one or more biodegradable linkages.

41. The hydrogel drug depot of claim 40, wherein said biodegradable linkage is an ester linkage.

42. The hydrogel drug depot of claim 38, wherein said controlled release kinetic profile comprises an initial bolus release profile followed by a release profile selected from the group consisting of zero order, pseudo zero order, and first order.

43. A kit for forming a hydrogel drug depot, said kit comprising an agent of interest selected from the group consisting of a therapeutic agent and a diagnostic agent, a first polymer having two or more thiol groups, and a second polymer having two or more vinyl sulfone groups, wherein said first polymer and said second polymer are capable of covalently bonding to one another under physiological conditions to form a thioether cross-linked hydrogel matrix so as to entrap said agent of interest therein.

44. The kit of claim 43, wherein said physiological conditions is in vivo.

45. The kit of claim 43, wherein said hydrogel matrix comprises polyalkylene oxide.

46. The kit of claim 45, wherein said polyalkylene oxide is a homopolymer, copolymer or combination thereof of polyethylene glycol or derivative thereof.

47. The kit of claim 46, wherein polymer matrix comprises a controlled release kinetic profile characterized by release of a therapeutically effective amount of said therapeutic agent from said thioether cross-linked hydrogel matrix over a time course of three or more days.

48. The kit of claim 47, wherein said time course is five or more days.

49. The kit of claim 47, wherein said time course is ten or more days.

50. The kit of claim 47, wherein said time course is fifteen more days.

51. The kit of claim 47, wherein said time course is twenty or more days.

52. The kit of claim 47, wherein said controlled release kinetic profile comprises an initial bolus release profile followed by a release profile selected from the group consisting of zero order, pseudo zero order, and first order.

53. The kit of claim 43, wherein said therapeutic agent is selected from the group consisting of small organic molecule, nucleic acid, peptide, polypeptide, protein, carbohydrate, vaccine, adjuvant, and lipid.

54. The kit of claim 43, wherein said diagnostic agent is selected from the group consisting of a contrast/imaging agent, radionuclide, chromophore, fluorophore and non-radioactive isotope.

55. The kit of claim 43, wherein said first polymer comprises a molecular weight of 200 to 20,000 Daltons.

56. The kit of claim 43, wherein said second polymer comprises a molecular weight of 100 to 5,000 Daltons.

57. The kit of claim 43, wherein said first polymer comprises between 2 and 20 thiol groups.

58. The kit of claim 43, wherein said first and second polymers are provided in preformed

aliquots for admixing to generate a defined molar ratio of said first and second polymers for controlling the controlled release kinetic profile of said hydrogel drug depot.

59. A method of producing a kit according to claim 43, said method comprising: assembling in said kit an agent of interest selected from the group consisting of a therapeutic agent and a diagnostic agent, a first polymer having two or more thiol groups, and a second polymer having two or more vinyl sulfone groups, wherein said first polymer and said second polymer are capable of covalently bonding to one another under physiological conditions to form a thioether cross-linked hydrogel matrix so as to entrap said agent of interest therein.

60. The method of claim 59 wherein said hydrogel matrix comprises polyalkylene oxide.

61. A method for preparing a matrix physically entrapping at least one therapeutic agent comprising the steps of

(a) preparing a solution comprising said at least one therapeutic agent and at least one polymer on which at least two thiol groups are present; and

(b) incubating said solution under conditions that cause cross-linking of thiol groups of said polymer to form a cross-linked matrix physically entrapping said at least one therapeutic agent.

62. The method of claim 1 wherein said matrix has at least one controlled release in-vivo kinetic profile.

63. The method of claim 1 wherein said therapeutic agent is a small-molecule drug, protein, peptide, polysaccharide, or polynucleotide.

64. The method of claim 63 wherein said protein is selected from the group consisting of erythropoietin, α-interferon and growth hormone.

65. The method of claim 63 wherein said protein is a recombinant protein.

66. The method of claim 63 wherein said therapeutic agent is a polysaccharide.

67. The method of claim 66 wherein said polysaccharide is a sulfated polysaccharide.

68. The method of claim 67 wherein said sulfated polysaccharide is heparin or calcium spirulan.

69. The method of claim 63 wherein said therapeutic agent is a polynucleotide.

70. The method of claim 69 wherein said polynucleotide is an antisense oligonucleotide.

71. The method of claim 61 wherein said therapeutic agent is a compound capable of entrapment in said matrix and controlled release in vivo therefrom.

72. The method of claim 61 wherein said therapeutic agent is derivatized to increase its molecular weight.

73. The method of claim 62 wherein said derivatization is selected from the group consisting of polymerization and conjugation to poly(ethylene glycol).

74. The method of claim 61 wherein said cross-linking of said polymer on which at least two thiol groups are present comprises disulfide bonds, thioether bonds, or combinations thereof.

75. The method of claim 61 wherein said polymer on which at least two thiol groups are present is a homopolymer or a copolymer.

76. The method of claim 61 wherein said polymer is selected from the group consisting of poly(ethylene glycol), carboxymethylcellulose, dextran, polyvinyl alcohol, N-(2-hydroxypropyl)methacrylamide, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, polypropylene oxide, copolymers of ethylene/maleic anhydride copolymer, polylactide/polyglycolide copolymers, polyaminoacids, copolymer of polyethylene glycol and an amino acid, and polypropylene oxide/ethylene oxide copolymers.

77. The method of claim 76 wherein said polymer is derived from a poly(ethylene glycol) (PEG) derivative selected from the group consisting of α,ω-dihydroxy-PEG and α,ω-diamino-PEG.

78. The method of claim 77 wherein said polymer is prepared from α,ω-diamino-poly(ethylene glycol) and thiomalic acid; α,ω-dihydroxy-poly(ethylene

glycol) and thiomalic acid; or α,ω-dicarboxy-PEG subunits and lysine, wherein free carboxy groups on lysine residues are derivatized to provide thiol groups.

79. The method of claim 76 wherein said poly(ethylene glycol) subunit size is from about 200 to about 20,000 Da.

80. The method of claim 79 wherein said poly(ethylene glycol) subunit size is from about 600 to about 5,000 Da.

81. The method of claim 61 wherein said polymer has from 2 to about 20 thiol groups.

82. The method of claim 61 wherein said polymer has from about 3 to about 20 thiol groups.

83. The method of claim 82 wherein said polymer has from about 3 to about 8 thiol groups.

84. The method of claim 61 wherein said thiol groups on said polymer are sterically hindered.

85. The method of claim 61 wherein said conditions that cause cross-linking of said thiol groups comprises reaction in the presence of an oxidizing agent or reaction with a cross-linking agent.

86. The method of claim 85 wherein said oxidizing agent is selected from the group consisting of molecular oxygen, hydrogen peroxide, dimethylsulfoxide, and molecular iodine.

87. The method of claim 85 wherein said cross-linking agent is a bifunctional disulfide-forming cross-linking agent or a bifunctional thioether-forming cross-linking agent.

88. The method of claim 85 wherein said cross-linking agent is a polymeric cross-linking agent.

89. The method of claim 88 wherein said polymeric cross-linking agent has a molecular weight of about 300 to about 5,000 Da.

90. The method of claim 87 wherein said cross-linking agent is selected from the group consisting of 1,4-di-[3',2'-pyridyldithio(propionamido)butane]; α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol); α,ω-divinylsulfone-poly(ethylene glycol); and α,ω-diiodoacetamide-poly(ethylene glycol).

91. The method of claim 61 wherein said matrix is provided in a form selected from the group consisting of a gel, microparticles, and nanoparticles.

92. The method of claim 1 wherein said solution comprises at least one additional polymer comprising thiol groups.

93. A method for the controlled release of a therapeutic agent in an animal comprising administration to said animal a therapeutically effective amount of said therapeutic agent in a matrix prepared in accordance with claim 61.

94. The method of claim 93 wherein said matrix is administered by a route selected from the group consisting of subcutaneous, oral, intravenous, intraperitoneal, intradermal, subdermal, intratumor, intraocular, intravisceral, intraglandular, intravaginal, intrasinus, intraventricular, intrathecal, intramuscular, and intrarectal.

95. A method for the controlled release of a therapeutic agent under at least two different in-vivo kinetic controlled release profiles in an animal comprising administration to said animal a therapeutically effective amount a mixture of at least two matrices prepared in accordance with the method of claim 61, each matrix having a different controlled release in-vivo kinetic profile.

96. The method of claim 93 wherein said controlled release occurs as a consequence of diffusion from said matrix or biodegradation of said matrix by an in-vivo degradation pathway selected from the group consisting of reducing agents, reductases, S-transferases, esterases, peptidases, proteases, non-enzymatic hydrolysis, and thioesterases.

97. A method for the controlled release of a therapeutic agent in an animal comprising administration to said animal a therapeutically effective amount of said therapeutic agent in a matrix prepared in accordance with claim 61, wherein said matrix is formed

immediately prior to or during administration to said animal.

98. A pharmaceutical composition consisting of a matrix comprising a therapeutic agent exhibiting at least one first controlled release in-vivo kinetic profile, said matrix comprising at least one cross-linked polymer on which at least two thiol groups are present, said cross-linked polymer physically entrapping said therapeutic agent.

99. The pharmaceutical composition of claim 98 wherein said matrix has at least one second controlled release in-vivo kinetic profile.

100. The method of claim 98 wherein said therapeutic agent is a small-molecule drug, protein, peptide, polysaccharide, or polynucleotide.

101. The method of claim 100 wherein said protein is selected from the group consisting of erythropoietin, α-interferon and growth hormone.

102. The method of claim 100 wherein said protein is a recombinant protein.

103. The method of claim 100 wherein said therapeutic agent is a polysaccharide.

104. The method of claim 103 wherein said polysaccharide is a sulfated polysaccharide.

105. The method of claim 104 wherein said sulfated polysaccharide is heparin or calcium spirulan.

106. The method of claim 100 wherein said therapeutic agent is a polynucleotide.

107. The method of claim 106 wherein said polynucleotide is an antisense oligonucleotide.

108. The pharmaceutical composition of claim 98 wherein said therapeutic agent is a compound capable of entrapment in said matrix and controlled release therefrom.

109. The pharmaceutical composition of claim 98 wherein said therapeutic agent is derivatized to increase its molecular weight.

110. The pharmaceutical composition of claim 109 wherein said derivatization is selected from the group consisting of polymerization or conjugation to poly(ethylene glycol).

111. The pharmaceutical composition of claim 98 wherein said cross-linking of said polymer on which at least two thiol groups are present comprises disulfide bonds, thioether bonds, or combinations thereof.

112. The pharmaceutical composition of claim 98 wherein said polymer on which at least two thiol groups are present is a homopolymer or a copolymer.

113 The pharmaceutical composition of claim 98 wherein said polymer is selected from the group consisting of poly(ethylene glycol), carboxymethylcellulose, dextran, polyvinyl alcohol, N-(2-hydroxypropyl)methacrylamide, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, polypropylene oxide, copolymers of

ethylene/maleic anhydride copolymer, polylactide/polyglycolide copolymers, polyaminoacids, copolymer of polyethylene glycol and an amino acid, and polypropylene oxide/ethylene oxide copolymers.

114. The pharmaceutical composition of claim 113 wherein said polymer is derived from a poly(ethylene glycol) (PEG) derivative selected from the group consisting of α,ω-dihydroxy-PEG and α,ω-diamino-PEG.

115. The pharmaceutical composition of claim 114 wherein said polymer is prepared from α,ω-diamino-poly(ethylene glycol) and thiomalic acid; α,ω-dihydroxy-poly(ethylene glycol) and thiomalic acid; or α,ω-dicarboxy-PEG subunits and lysine, wherein free carboxy groups on lysine residues are derivatized to provide thiol groups.

116. The pharmaceutical composition of claim 115 wherein said poly(ethylene glycol) subunit size is from about 200 to about 20,000 Da.

117. The pharmaceutical composition of claim 115 wherein said poly(ethylene glycol) subunit size is from about 600 to about 5,000 Da.

118. The pharmaceutical composition of claim 98 wherein said polymer has from 2 to about 20 thiol groups.

119. The pharmaceutical composition of claim 118 wherein said polymer has from about 3 to about 20 thiol groups.

120. The pharmaceutical composition of claim 119 wherein said polymer has from about 3 to about 8 thiol groups.

121. The pharmaceutical composition of claim 98 wherein said thiol groups on said polymer are sterically hindered.

122. The pharmaceutical composition of claim 98 wherein said conditions that cause cross-linking of said thiol groups comprises reaction in the presence of an oxidizing agent or reaction with a cross-linking agent.

123. The pharmaceutical composition of claim 122 wherein said oxidizing agent is selected from the group consisting of molecular oxygen, hydrogen peroxide, dimethylsulfoxide, and molecular iodine.

124. The pharmaceutical composition of claim 122 wherein said cross-linking agent is a bifunctional disulfide-forming cross-linking agent, or reaction with a bifunctional thioether-forming cross-linking agent.

125. The pharmaceutical composition of claim 122 wherein said cross-linking agent is a polymeric cross-linking agent.

126. The pharmaceutical composition of claim 125 wherein said polymeric cross-linking agent has a molecular weight of about 300 to about 5,000 Da.

127. The pharmaceutical composition of claim 124 wherein said cross-linking agent is selected from the group consisting of 1,4-di-[3',2'-pyridyldithio(propionamido)butane]; α,ω-di-O-pyridyldisulfidyl-poly(ethylene glycol); α,ω-divinylsulfone-poly(ethylene glycol); and α,ω-diiodoacetamide-poly(ethylene glycol).

128. The pharmaceutical composition of claim 98 wherein said matrix is provided in a form selected from the group consisting of a gel, microparticles, and nanoparticles.

129. The pharmaceutical composition of claim 98 wherein said solution comprises at least one additional polymer comprising thiol groups.

130. The pharmaceutical composition of claim 98 wherein said polymer or cross-linking agent additionally comprises a functional group.

131. The pharmaceutical composition of claim 130 wherein said functional group is derivatized with a label.

132. The pharmaceutical composition of claim 131 wherein said label is a contrast/imaging agent, radionuclide, chromophore, fluorophore, or nonradioactive isotope.

133. The pharmaceutical composition of claim 131 wherein said label is a metabolically stable polymer component that after degradation of the polymer is detectable in the urine.

FIGURE 1 of 2

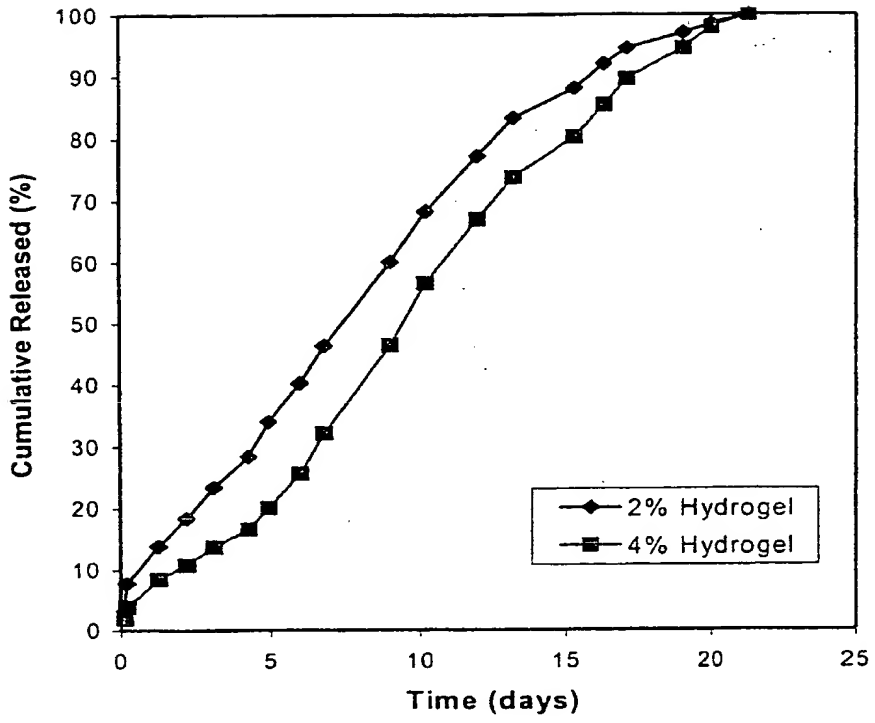

Figure 1. Release of fluorescein-BSA from thiol containing polymer hydrogel in PBS, pH 7.4 at 25 degree C.

FIGURE 2 of 2

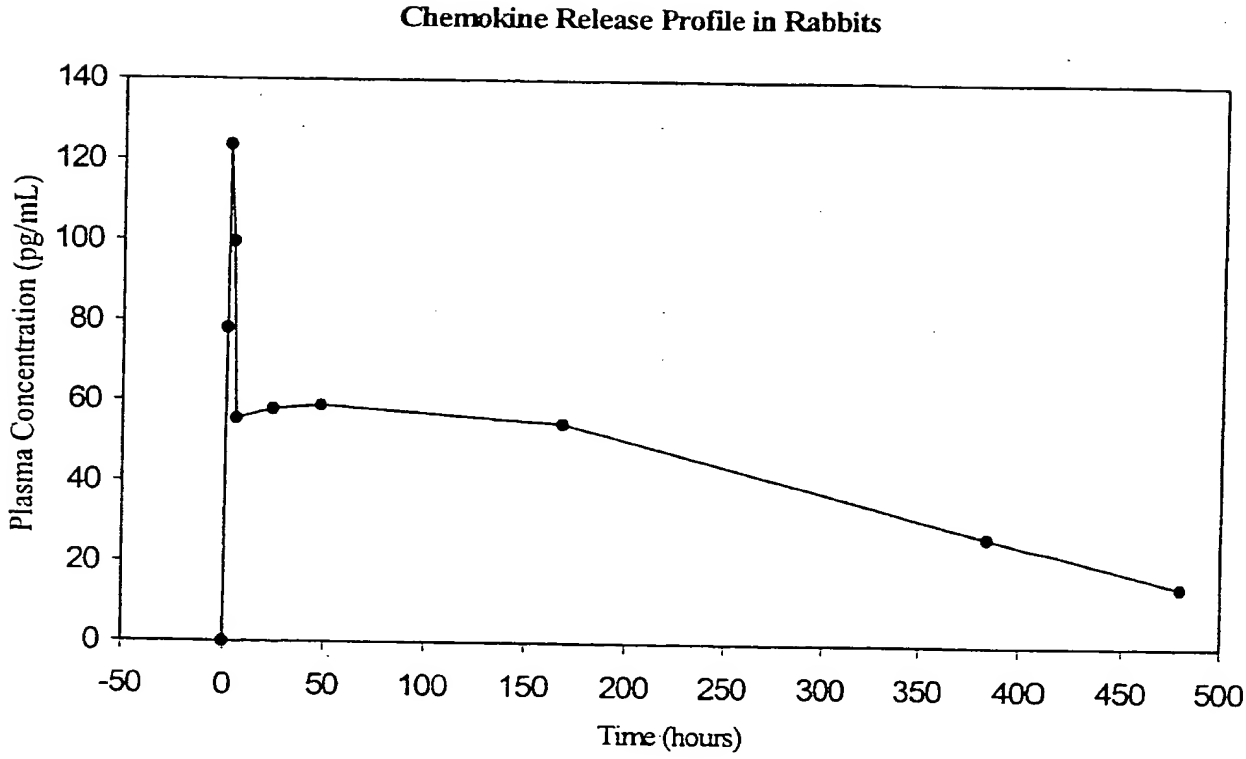

Figure 3. Release profile of chemokine from thiol containing polymer hydrogel in rabbits.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/16881

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K47/10 A61K47/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 874 500 A (RHEE WOONZA M ET AL) 23 February 1999 (1999-02-23) column 1, line 13 - line 20 column 3, line 36 - line 43 -/--	1-7, 9-20, 22-32, 35-40, 42-53, 57-63, 66-68, 71, 74-76, 81,85, 88,91, 93, 96-100, 103-105, 108, 111-113, 118,128

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	18/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Marttin, E

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/16881

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	column 4, line 38 -column 5, line 32 column 6, line 33 - line 42 column 6, line 62 column 7, line 2 - line 4 column 8, line 60 - line 63 column 13, line 37 -column 14, line 28 column 14, line 46 -column 15, line 63; claims 1,7,9-12,15 column 2, line 26 - line 30	
X	WO 96 40829 A (VIVORX PHARMACEUTICALS INC ;SOJOMIHARDJO SOEBIANTO A (US); DESAI N) 19 December 1996 (1996-12-19) page 3, last paragraph -page 4, paragraph 1 page 7, paragraph 3 -page 8, paragraph 5 page 10, line 3-6 page 17, paragraph 2 -page 17, last paragraph page 19, last paragraph -page 22, line 30; claims 1,2,7,15; examples 2,3	61,62, 71,74, 76,91, 93,94, 96-98, 112,128
X	US 4 438 258 A (GRAHAM NEIL B) 20 March 1984 (1984-03-20) column 1, line 4 - line 9 column 4, line 40 - line 43 column 5, line 37 - line 40 column 6, line 6 - line 26 column 6, line 49 - line 62 column 7, line 4 - line 10 column 8, line 39 -column 9, line 14 column 10, line 66 -column 11, line 8 column 11, line 31 -column 12, line 33 column 12, line 54 - line 57; claims 1,2,11-18,22,29	22,35-37
A	WOGHIREN C ET AL: "PROTECTED THIOL-POLYETHYLENE GLYCOL: A NEW ACTIVATED POLYMER FOR REVERSIBLE PROTEIN MODIFICATION" BIOCONJUGATE CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY, WASHINGTON, vol. 4, no. 5, 1 September 1993 (1993-09-01), pages 314-318, XP000395050 ISSN: 1043-1802 abstract page 315, paragraph 1 page 315, last paragraph page 318, paragraph 1	1-133

-/—

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/16881

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 446 090 A (HARRIS J MILTON) 29 August 1995 (1995-08-29) column 3, line 47 - line 54 column 4, line 11 - line 21 column 8, line 28 - line 35 column 9, line 6 - line 22; claims 1-11; example 2	1-60
A	ZHAO X ET AL: "NOVEL DEGRADABLE POLY(ETHYLENE GLYCOL) HYDROGELS FOR CONTROLLED RELEASE OF PROTEIN" JOURNAL OF PHARMACEUTICAL SCIENCES,US,AMERICAN PHARMACEUTICAL ASSOCIATION. WASHINGTON, vol. 87, no. 11, 1 November 1998 (1998-11-01), pages 1450-1458, XP000783392 ISSN: 0022-3549 the whole document	1-133
T	"DPDPB 1,4-Di-'2'-pyridyldithio)propionamido!butane" PIERCE, 'Online! pages 1-3, XP002149390 Rockford Retrieved from the Internet: <URL:http://63.111.197.2/Lib/ViewDoc.cfm?doc=654> 'retrieved on 2000-10-05! page 1, paragraph 1	61,85, 87,90, 98,122, 124,127

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 00/16881

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5874500 A	23-02-1999	US 6051648 A	18-04-2000
		AU 717660 B	30-03-2000
		AU 1334497 A	14-07-1997
		EP 0876165 A	11-11-1998
		JP 2000502380 T	29-02-2000
		WO 9722371 A	26-06-1997
WO 9640829 A	19-12-1996	AU 5801296 A	30-12-1996
US 4438258 A	20-03-1984	DE 3280021 D	21-12-1989
		DK 260782 A	13-12-1982
		EP 0067671 A	22-12-1982
		GB 2108517 A,B	18-05-1983
		JP 1793659 C	14-10-1993
		JP 4080052 B	17-12-1992
		JP 58005316 A	12-01-1983
		JP 2027257 C	26-02-1996
		JP 5229963 A	07-09-1993
		JP 7053672 B	07-06-1995
		US 4528365 A	09-07-1985
		US 4584188 A	22-04-1986
US 5446090 A	29-08-1995	AU 687937 B	05-03-1998
		AU 1054895 A	29-05-1995
		BG 100568 A	31-12-1996
		BR 9408048 A	24-12-1996
		CA 2176203 A	18-05-1995
		CN 1137280 A	04-12-1996
		CZ 9601375 A	16-10-1996
		EP 0728155 A	28-08-1996
		FI 962004 A	10-05-1996
		JP 9508926 T	09-09-1997
		KR 225746 B	15-10-1999
		NO 961918 A	12-07-1996
		NZ 276313 A	24-04-1997
		PL 314298 A	02-09-1996
		SK 60896 A	09-04-1997
		US 5900461 A	04-05-1999
		WO 9513312 A	18-05-1995
		US 5739208 A	14-04-1998